IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Dos Santos, et al.	)	Examiner: Cordero Garcia, M. M.
Serial No: 10/563,276	)	Art Unit: 1654
Filed: January 4, 2006	)	Attny Docket: VA/H-33271A
For: Protein from photobacterium damselae and uses thereof	)	Confirmation No.: 4311

Commissioner for Patents
Post Office Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF ANDREW C. BARNES UNDER 37 C.F.R. §1.132

Sir:

I, Andrew C. Barnes, hereby declare that:

1. I have reviewed and am familiar with the contents of the above-referenced patent application (the "`276 application").

2. I am the sole named inventor on International Publication No. WO 01/10459 (the "'459 publication"), which is the prior art reference being asserted against the claims of the `276 application by the U.S. Patent Office.

3. I received my Ph.D. degree from The University of Edinburgh Medical School in 1993. Since 1992, I have held various positions in the area of immunology and infectious disease of aquatic species, including, from 1992-1993 as a Higher Scientific Officer at DAFS Marine Laboratory in Aberdeen, Scotland; 1993 as a Post Doctoral researcher at Moredun Animal Health in Edinburgh, Scotland; from 1993-1999 as a Senior Research Scientist at Aqua Health (Europe) Ltd. in Aberdeen, Scotland; and from 1999-2003 as a Senior Research Scientist at Novartis Animal Health, based in Aberdeen. Since 2003, I have been a member of the Science Faculty at The University of Queensland in Brisbane, Australia. Currently, I am an Associate

Professor of Aquatic Animal Health in the School of Biological Sciences at The University of Queensland and have an active research program related to vaccines and environmental factors affecting the immune system in fish and marine invertebrates, specifically as they relate to infectious disease. I have published about 60 papers (peer-reviewed and invited) and book chapters in this area.

4. At the time that I was doing the work disclosed in the `459 publication, I was an employee of Aqua Health Ltd., a company which later became part of Novartis Animal Health. During that time, I worked with and became very familiar with the work being conducted by Drs. Nuno Miguel Simões Dos Santos and Ana Maria Silva Do Vale, who are two of the inventors on the `276 application. Indeed, Dr. Dos Santos was appointed as a post-doctoral researcher by Aqua Health (Europe) on my recommendation following collaborative work between myself and Dr. Dos Santos during his PhD studies at Wageningen Agricultural University, Netherlands. At that time, Dr. Do Vale was also a PhD student, working with Dr. Dos Santos at the University of Porto, Portugal, using Photobacterium challenge models in sea bass developed by Dr. Dos Santos for testing Aqua Health vaccines in order to investigate interactions with sea bass peritoneal leukocytes.

5. In the `459 publication, I describe a protein that I identified from the bacterium, *Photobacterium damselae* subsp. *piscicida*, that is likely to be involved in invasion and internalization of the bacterium into cells of marine fish in which it colonizes. At the time I filed the application that led to the '459 publication, the protein disclosed therein was thought to have a molecular mass close to, but less than 55KDa. We now know that the protein is actually 52KDa (see Paragraph 7 of this Declaration).

6. The `276 application discloses a 55KDa protein from *P. damselae*, which, in its native form, is toxic to fish cells due to its ability to initiate apoptosis. It was during the investigations using the challenge models that Dr. Do Vale discovered that peritoneal leukocytes were becoming apoptotic. This work was published in 2003 in volume 15 of Fish & Shellfish Immunology (pp. 129-144), a copy of which is enclosed with this document, and led to subsequent investigation of the cause of apoptosis in these cells during infection.

7. Based on my review of the '276 application, my knowledge of the work done by Drs. Dos Santos and Do Vale, and my own work, the *P. damselae* protein that I describe in the '459 publication is NOT the same protein disclosed by the '276 application.

First, the two proteins have different masses. Research that I have published along with Drs. Dos Santos and A. E. Ellis in 2005 in volume 121 of Developments in Biologicals (Basel, Karger, pp. 75-84), a copy of which is enclosed with this document, describes further work on the same protein described in the '459 publication and refers to this protein as having a mass of 52KDa. This is a more accurate statement of the protein's molecular mass because it is based on additional experience in working with this protein, including use of size exclusion chromatography (gel filtration) to determine native molecular mass. Prior determinations of mass of this protein were based on migration of the protein in SDS-PAGE gels.

Further, as my Dev. Biol. paper, above, further shows, the 52KDa protein is glycosylated, based on its ability to be stained by lectins and, as already mentioned, appears to function in invasion and internalization of the bacterium into host cells, i.e. it is a putative "*invasin*." In addition, injecting fish with the 52KDa protein during experiments conducted at VESO Vikan, Namsos, Norway, even at high doses (250 µg/fish), was shown to be non-toxic to fish. This work on the 52KDa protein was conducted under a European Commission grant to enable use of Large Scale Facilities and the results were documented in the report submitted to the Commission at the completion of this study.

Additionally, expression of the 52KDa protein by *P. damselae* is significantly increased by excess iron in the growth medium. Finally, the 52KDa protein was also shown to be N-terminal blocked, so it was not possible to obtain the N-terminal amino acid sequence of this protein.

In contrast to the 52 KDA protein described in the '459 publication and the Dev. Biol. paper, the protein described in the '276 application is larger. This protein is 55KDa, does not appear to be glycosylated, and has apoptotic activity for fish cells. Indeed, injection of fish with the purified protein in native form is lethal.

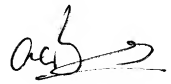
Additionally, expression of the 55KDa protein by *P. damselae* is not dependent on iron in the growth medium but, instead, is maximally expressed in mid-logarithmic growth of the bacterium. Finally, the N-terminus of the 55KDa protein is not blocked, as indicated by the

ability to obtain the N-terminal amino acid sequence of the protein, which is also described in the '276 application.

Therefore, it is a scientific fact that the 52KDa protein disclosed by the '459 publication is distinct from the 55KDa protein disclosed by the '276 application.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully Submitted



Date: 14th January 2010

Andrew C. Barnes